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(54)【発明の名称】　核酸の単離方法

(57)【要約】
本発明は、サンプルを界面活性剤および固体支持体に接触させ、これによって上記サンプル中の可溶性核酸がこの支持体に結合し、次いでこの支持体を結合した核酸とともに上記サンプルから分離することからなる、サンプルから核酸を単離する方法を提供する。本発明の方法を、DNA を単離するのに用いる場合、同一のサンプルから RNA を単離する追加工程と都合良く組み合わせることができる。

RNA

DNA

RNA

0.2 30% w/v

RNA

DNA RNA

DNA RNA

cDNA

DNA RNA

DNA RNA

RNA RNA

DNA

DNA RNA

RNA RN

RNA

RNA mRNA cDNA

RNA DNA DNA

RNA DN

A RNA DNA RN

RT-PCR

DNA R

NA LiCl

RNA

DNA

RNA mRNA m

RNA RNA RNA

mRNA

mRNA 50 300 A

mRNA (polyadenylated)

(A)⁺mRNA RNA 95 % R

NA RNA A

A

RNA

(dT)-

mRNA

dT

mRNA

$(A)^+$RNA

mRNA

RNA

$(A)^+$RNA

microfuge

RNA LiCl

LiDS/SDS dT

-K

mRNA 15 mRNA 30

mRNA

mRNA

mRNA

mRNA

GTC sarkosyl

GTC- RN

4M GTC

DNA

US-A-5,234,8

06

RNA PCR

PCR

RNA DNA

DNA RNA

DNA DNA

cDNA

DNA

RNA

RNA

PCR

(Banerjee S K et al. 1995 Biotechniques 18:769-773)

20

0.5

(SDS)

0.2 30 % 0.5 30 %

0.5 15 % 10 %

1.0 % 0.5 %

0.1

M 250 500 mM

50 mM

mEDTA EGTA

10 mM DTT -

100mM -HCl PH 7.5

10mM EDTA

2% SDS

100mM Cl pH 7.5

10mM EDTA

5% SDS

10mM NaCl

100mM Cl pH 7.5

500mM LiCl

10mM EDTA

1% LiDS

DNA-

m

m 10 m

m 2.8 m 4

.5 m

5%

US-A-4336173

Qiagen Pharmacia

Serotec Dyno Particles AS Lillestr m Norway

Sintef EP-A-106873 D

YNABEADS Dynal AS Oslo Norway

4,336,173 4,459,378

4,654,267

DNA RNA DNA

DNA

10mM -HC1 pH 8.0/10mM NaC1

PCR

DNA

DNA

DNA

65 10

PCR

DNA

RNA DNA DNA

RNA NaOH RNA

DNA

RNA RNA

DNA

DNA RNA

DNA

RNA RNA

RNA

RNA RNA

DNA RNA

DNA RNA

DNA RNA

D

NA LiCl RNA GTC

RNA

DNA

DNA

DNA

RNA

RNA

DNA

RNA

RNA DNA

RNA RNA RNA

RNA dT

____ DNA

OD nM 0.100

0.427 257.6 nM 0.292 236.4 n

M

____ DNA

Hind III

____ PCR PCR

Hind III PCR

____ PCR Hin

d III

Hind III

____ DNA Dynabeads DNA DIRECT

DNA 10 1

DNA Dynabeads DNA DIRECT

Dynabeads DNA DIRECT

DNA

Hind III

II Dynabeads DNA DIRECT DNA

Dynabeads DNA DIRECT DNA 20 g

AMXY PCR

DNA 200 ng AMXY PCR

____ Dynabeads DNA DIRECT

Dynabeads DNA DIRECT 10 1

DNA 10% DNA

PCR 20% Hind

III M 100 bp L

____

EDTE

Dynabeads DNA DIRECT

A B 10 L

DNA

1 DNA 10% DNA

PCR 20%

20% DNA

EDTA Dynabeads DNA DIRECT

10 1 DNA

10% DNA PCR 20%

II

Dynabeads DNA DIRECT

10 1

DNA 10% DNA

PCR 20%

Dynabeads DNA DIRECT

1 Dynabeads

DNA DIRECT A B

DNA 10% DNA

PCR 20% II 10^5

Daudi Dynabeads DNA DIRECT

DNA 120 1 DNA 1

PCR 20%

DNA Hind III PCR

100 bp

10

Dynabeads DNA DIRECT

A DNA

DIRECT DNA PCR 20%

M 100 bp B

20ng DNA C

PCR

11 mRNA Dynabeads DNA DIRECT mRNA Dynabe

ads DNA DIRECT DNA Dynabeads Oligo(dT)25

100 DNA DIRECT Dynabeads

DNA mg

mg mg

10mg mRNA

DNA

DNA

RNA

12 (A) (B) (C) (D)

DNA PCR

DNA 200 1 DNA DIRECT 20%

DNA 10% RPC

PCR 2.5% DNA

5% 16S rRNA DNA

DNA

DNA 18S rDNA trn

L B15C

DNA

PCR

DNA

4 x 10^6 HL 60 PBS

10 1 PBS 0.1 ml 5% SDS/10 mM

TrisCl pH 8.0/1 mM EDTA] に再懸濁させたトシル活性化 Dynabeads® M-

280 DYNAL A/S

ーブすることにより得られる 1 mg の Dynabeads® M-280* を加えた。これに、

ml
トし、その後 DNA を結合した Dynabeads® を、磁石に引き付け、液相を除

ml 50mM NaCl/10mM TrisCl pH 8.0/

1mM EDTA DNA 0.1 ml

65

DNA

DNA OD_{260} /OD_{280} 1.72

TE DNA 1.7 1.9 DNA

OD_{260}

50 g/ml OD_{260} =1.0 OD_{260} 0.436 0

.1ml 10 mm 2.18 g DNA

DNA 2.67 g 82%

DNA >20 kb

表 1

PERKIN-ELMER LAMBDA BIO UV/VIS 分光器
アプリケーション番号 3 : 2 6 0 / 2 8 0 NM比

試 料	サイクル	波 長	データ	単 位
	15:50	オートゼロ		
004	15:56	260.0 nm	0.436	ABS
		280.0 nm	0.253	ABS
		比	1.723	RAT

DNA

1 EDTA 50 1 5% SDS 1 PBS

の 50 μg の Dynabeads® M-280* を加えた。この溶解物を、1 分室温でイン

0.5 ml TrisCl pH 7.5

DNA

0.5 ml 10mM TrisCl pH 7.5

DNA 40 1 TE 10mM TrisCl pH 8.0/1 mM EDTA

1 PCR GAPDH PCR

PCR

50 1 PCR 10 1

+++ DNA

溶解バッファ	洗浄バッファ	結 果
2% SDS	50 mM NaCl/1 x TE	+++
2% SDS/1 x TE	50 mM NaCl/1 x TE	+++
2% SDS/1 x TE/10 mM NaCl	50 mM NaCl/1 x TE	+++
5% SDS	50 mM NaCl/1 x TE	+++
5% SDS/1 x TE	50 mM NaCl/1 x TE	+++
5% SDS/1 x TE/10 mM NaCl	50 mM NaCl/1 x TE	+++
1% LiDS/10 x TE/0.5 M LiCl	50 mM NaCl/1 x TE	+++
1% LiDS/10 x TE/0.5 M LiCl	150 mM LiCl/1 x TE	+++
5% LiDS	150 mM LiCl/1 x TE	+++
5% SDS	150 mM LiCl/1 x TE	+++
1% サルコシル	150 mM LiCl/1 x TE	+++

1 x TE 10mM TrisCl pH 8.0/1 mM EDTA 10 X TE 100 mM TrisCl pH 8.0/10 mM EDTA

実施例1の操作を辿ると、未被膜 Dynabeads® M-450 (Dynal A/S, オスロ、

CD2 DNA

50 1 50 1 PB

S 150mM NaCl/10mM Na_2HPO_4 pH 7.4 10 1 4×10^6
の Dynabeads® M-450 Pan-T (CD2)(Dynal AS, オスロ、ノルウェーより入手

30

/

/ 200 1 PBS 200
g の Dynabeads® M-280* (同上) および 200μl の溶解バッファ [100mM

Tris-HCl pH 8.0/500mM LiCl/10mM EDTA pH 8.01/1% LiDS

DNA/

DNA/ 200 1 [10mM Tris-HCl

pH 8.0/150mM LiCl/1mM EDTA pH 8.0] 50 1

65

1 GAPDH PCR

DNA

DNA ml EDTA Dynabeads DNA

DIRECT Dynal A/S,

Dynabeads® M-280* と等価のビーズを含有したキット) を用いて、同じ血液

10 1 DNA 65

DNA Dynabeads

Dynabeads DNA DIRECT DNA

DNA 0.2%

DNA 10 1 (5ml 0

.2%)

DNA John John S.W.M. G Weitzner R Rose

n C.R.scriver 1991 A Rapid Procedure for Extracting Genomic DNA from Leukocytes Nucl Acid Res 19(2): 408

Dynabeads DNA DIRECT 200 1 Dynabeads DNA DIRECT 10 1 1.5 ml (/ 200 g Dynabeads) DNA Dynabeads

DNA/Dynabeads Dynal E(Magnetic Particle Collector E)(MPC-E) Dynal MPC 10 1 TE pH 8.0 65

DNA

DNA 1.5% 1 x TAE DS34 667 I 1 ( ) DNA ( ) Hind III 23.13 kb DNA 20 kb

DNA DIRECT

ACD DNA 10% DNA 200 ng X-Y (X-Y homologous amelogenin)(AMXY) (Akane A. K. Matsubara H Nakamura S Takahashi K Kimura 1994 Purification

of Highly Degraded DNA by Gel Filtration for PCR BioTechnigues 16(2): 235-238) amplicon PCR

DNA DIRECT DNA

PCR 50 1 10 x PCR Perkin Elmer 1 x dNTP Pharmacia 0.2mM (amplitaq) (Perkin Elmer pmol AMXY-1F 5'-CTGA TGGTTGGCCTCAAGCCT-GTG-3' AMXY-4R 5'-TTCATTGTAAGAGCAAAGCAAACA-3' PCR Perkin Elmer GeneAmp PCR System 9600 AMXY PCR 94 38 x[94 30 55 30 72] 72 10 50 1 PCR 10 1 1.5% 1 x TAE DS34 667 II X-Y DNA (Akane et al 1994) 908 bp X 719 bp Y II X Y

Dynabeads DNA DIRECT

DNA

DNA PCR DNA DIRECT DNA

DNA PCR DNA DIRECT DNA PCR 10

溶解／結合　バッファ：	0.5 M LiCl 1 % LiDS 0.1 M TrisCl pH 7.5 10 mM EDTA 5 mM ジチオトレイトール (DTT)
洗浄バッファ：	0.15 M LiCl 10 mM Tris-HCl pH 8.0 1 mM EDTA

DNA

Dynal AS

Dynabeads DNA DIRECT

DNA

A: 3.6 x 10^6 /ml B: 2.6 x 10^6 /ml

200 1 Dynabeads DNA DIRECT

1.5 ml

DNA Dynabeads

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

40 1 TE pH 8.0

PCR

10%

DAPDH amplicon PCR

PCR 50 10 x PCR (Perkin E

lmer) 1 x dNTP Pharmacia 0.2mM

(amplitaq) (Perkin Elmer)

pmol GAPDH-Forward(5'-ACAGTCCATGCCATCAC

TGCC-3') GAPDH-Reverse(5'-GCCTGCTTCACCACCTTCTTG-3')

PCR Perkin Elmer GeneAmp PCR System 9600 GAPDH

PCR 94 34 x[94 30 61 30

72] 72 10

DNA PCR 1.5%

50 1 10 1 D

NA 50% 1 x TAE

DS34 667

DNA

Dynabeads DNA DIRECT Dynal AS

EDTA

DNA

DNA 200 1 Dynabeads DNA DIRECT

1 10 1

1.5 ml

DNA Dynabeads

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

20 40 1 TE pH 8.0 40

1 20 1

10%(20%)

(DAPDH) amplicon PCR

PCR Dynabeads TE

PCR 50 1 10 x PCR

Perkin Elmer 1 x dNTP

(Pharmacia) 0.2mM (

amplitaq)(Perkin Elmer) pmol G

APDH-Forward(5'-ACAGTCCATGCCATCACTGCC-3') GAPDH-Reverse(5'-GCCTGCT

TCACCACCTTCTTG-3') PCR Perkin Elmer GeneAmp PCR S

ystem 9600 GAPDH PCR 94

34 x[94 30 61 30 72] 72 10

50 1 10 1 DNA 25%

50% DNA PCR

1.5%

1 x TAE DS34

667

1 PCR

DNA 20% 10 1

10%

(Panaccio M

. M Georgesz A.M Lew 1993 FoLT PCR: A Simple PCR Protocol fo
r Amplifying DNA Directly from Whole Blood BioTechniques 14(3): 238-243
)

DNA DIRECT ACD(II) CPD

DNA

Dynabeads DNA DIRECT Dynal AS

EDTA DNA

-

20 +4

DNA

200 1 Dynabeads DNA DIRECT

1.5 ml

DNA Dynabeads

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

40 1 TE pH 8.0 PCR

Dynabeads TE

10%

DAPDH amplicon PCR

DNA PCR

1.5% 50 l 10 l

DNA 50% 1 x TA

E DS34 667

I

+4 -20

Dynabeads DNA DIRECT

DNA

10 l

1.5 ml 40 l PBS

90 DNA Dynabeads DNA DIRECT

10%

DAPDH amplicon PCR

DNA PCR 1.5

% 50 l 10 l

DNA 50% 1 x TAE

DS34 667

II

DNA PCR

DNA

DNA

1

DNA DIRECT DNA

200 l Dynabeads DNA

DIRECT 1.5ml

DNA Dynabeads

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

40 l TE pH 8.0 PCR

Dynabeads TE

10% DAPDH

amplicon PCR

DNA PCR 1.5%

50 l 10 l D

NA 50% 1 x TAE

DS34 667

I

( )

I DNA

1 DNA DIRECT

DNA

1

1 10 PCR

DNA

4×10^5 Daudi cells DNA DIRECT

DNA 4×10^5

DNA Dynabeads DNA DIRECT ml

ml

DNA/Dynabeads 120 1 TE

120 1 1 D

APDH amplicon PCR

DNA PCR 1.5%

50 1 10 1 D

NA 10% 1 x TAE

DS34 667

II 120

PCR

DNA

Dynabeads DNA DIRECT Dynal AS

DNA

200 1 Dynabeads DNA DIRECT 1.5 ml

DNA Dynabeads DNA Dynabea

ds

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

10

1 Dynabeads

DAPDH amplicon PCR

PCR 1.5%

50 1 10 1

1 x TAE DS34

667

10 PCR

---

mRNA DNA DIRECT DNA

mRNA 100 0.75ml

/ DNA DIRECT Dynabeads

DNA-Dynabeads Dynal

MPC-E 10mg

DNA DIRECT DNA (11)

Dynal mRNA DIRECT

mg Dynabeads $(dT)_{25}$ Dy

nabeads mRNA

mRNA-Dynabead MPC-E

Li

DS 0.75ml

mRNA-Dynabead

LiDS

mRNA 20 1 mM Tri

s-HCl pH 7.5 65 Dyn

abeads 1.0%

11

EtBr- DNA rRNA

RNA

11 10 DNA mRNA

DYNAL mRNA

DNA DNA DNA DIRECT

DNA

溶解／結合　バッファ：	0.5 M LiCl 1 % LiDS 0.1 M TrisCl pH 7.5 10 mM EDTA 5 mM ジチオトレイトール (DTT)
ＬｉＤＳ含有洗浄バッファ：	0.15 M LiCl 0.1 % LiDS 10 mM Tris-HCl pH 8.0 1 mM EDTA
洗浄バッファ：	0.15 M LiCl 10 mM Tris-HCl pH 8.0 1 mM EDTA

DNA

PCR DNA

(E.Coli) Baceillus cereus LB

37 (Agrobacterium

tumefaciens) YEB 40 28 (Sambrook J et al

. 1989 Molecular Cloning: A Laboratory Manual 2nd ed. Cold Spring Ha

rbour Laboratory NY.) (Prochlo

rthrix) NIVA 14 18 20

Norwegian Institute of Water Research 1991

20 450,000 DNA

2% 14

mg 20mg

DNA

Saccharomyces cerevisiae

IMR (Epp

ley R et al. 1967 Exp Mar Biol Ecol 1 191-208)

Arabidopsis thaliana (Hordeum vulgare)

Perca fluvatilis

mg 30 100mg 100

400 mg DNA

DNA

foreceps

(Kontes Scientific

Instruments Vineland New Jersey USA)

DNA

Dynabeads DNA DIRECT Dynal AS

DNA 200 1 D

ynabeads DNA DIRECT / 200 g Dynabeads

1.5 ml

15 DNA Dynabeads

65 15

DNA/Dynabeads Dynal E(MPC-E)

Dynal MPC

40 1 TE pH 8.0

65

DNA

DNA 1.5%

1 x TAE DS34

667

溶解／結合バッファ: **0.5 M LiCl**
1% LiDS
0.1 M TrisCl pH 7.5
10 mM EDTA
5 mM ジチオトレイトール (DTT)

洗浄バッファ: **0.15 M LiCl**
10 mM Tris-HCl pH 8.0
1 mm EDTA

DNA DIRECT

/ - DNA

Sambrook J et al. 1989

A-5 Sigma Chemicals Co.

St Louis USA

DNA Scot O.R Bendich A.J. 1994 "Plant Molecular Biol

ogy Manual" page D1: 1-8 Kluwer Academic Publisher Belgium

PCR

DNA DNA

PCR DNA PC

R PCR

15 pmol 200 M dNTP 10mM Tris-HCl pH 8.8 1

.5 mM $MgCl_2$ 50 mM KCl 0.1% Triton X-100 DynaZyme

Finnzymes Oy Finland 0.1-5 1 DN

A 50 1 PCR Perkin Elmer GeneAmp PCR Sy

stem 9600

amplicon

PCR 94-97 DNA 72

amplicon IUD

Brosius J. et al. 1978 Proc Natl Acad Sci. USA 57 4801

-4805 E Coli 334 939 16S rRNA

プライマー: CC 5'-TGTAAAACGACGGCCAGTCCAGACTCCTACGGGAGGCAGC-3'
CD 5'-CTTGTGCGGGCCCCCGTCAATTC-3'

CC -21 M13 5

DNA : 96 1

5 70 30

18S rRNA Medlin et al. 1990 Gene 71 491-499

A B

94 30 50 72 35

18S rRNA 600 bp. White et al. 1990 Innis M.A et al.

"PCR Products a Guide to Methods and Applications" page 315-32

2 Academic Press New York NS3 NS4

94 30 53 30 72

94 30 50 30 15

72 25

tRNA I Fangan et al. 1994 BioTechn

iques 16 484-494 C D

94 30 55 30 72 30

Arabidopsis thaliana BI5C 800 bp

5'-CGGGATCCCTAGGAGACACGGTGCCG-3' および
5'-GGAATTCGATCGGCGGTCTTGAAAC-3'

94 30 59 30 72 35

Barley B15C 800 bp

5'-CGGATCCCGTCATCCTCTTCTCGCACCCC-3' および
5'-GGAATTCCCTTCTTGGAGGGCAGGTCGGCG-3'.

94 30 60 30 72 35

(Perch)

D- 800-900 bp Hoelzel et al. 1

991 Mol Biol Evol. 8 475-493 HV2

5'-GGTGACTTGCATGTGTAAGTTCA-3'.

96 52 72 30

1.5%

1 x TAE DS34

667

12

_____

100-1000 ng

DNA (12A)

65 15

DNA 500 ng mg

0.25% DNA

_____

DNA 100-200 ng

300-500ng (12B)

DNA 5

% DNA PCR (12B)

0.5-5% DNA PCR

_____

DNA DIRECT

200-400 ng DNA (12C)

PCR DNA DNA PCR

5% DNA

_____

DNA

65 15

(12D) PCR DNA DNA

PCR 5% DNA

_____

300-500 ng DNA

( ) DNA 5

% DNA

PCR Hultman et

al. 1989 Nucleic Acids Res. 17 4937-4946

表　2：異なる器官からのＤＮＡ単離およびＰＣＲ増幅

種[a]		試料[b]	DNA 収量[c]	アンプリコン[d] Gen.	Org.	PCR 生成物[e]
バクテリア						
Bacillus sereus AH 75[e]	グラム陽性	新鮮	+++	16S		+++
E. coli NovaBlue[f]		新鮮	+++	16S		+++
A. tumefaciens GV 3105[g]		新鮮	+++	16S		+++
Planktothrix agardhii N-C 29	グラム陰性	新鮮	+++	16S		+++
P. prolifica N- C320		新鮮	+++	16S		+++
Microcystis aeruginosa N-C 43		新鮮	+++	16S		+++
M. aeruginosa N-C 228/1	シアノバクテリア	凍結	+++	16S		++
Anabaena bory N-C 246		凍結	+++	16S		++
Phormidium sp N-C 177		凍結	+++	16S		+++
Aphanizomenon sp N-C 103		凍結	++	16S		+++
P. hollandica N 5/89	プロクロロトリクス					
菌類						
Cortinarius sanguineus	担子菌類	d.fruitb.	+++	18S		+++
Cortinarius genitilis		d.fruitb.	nt	18S		+++
Russula integra		f.fruitb.	++	18S		+++
Laccaria bicolor		f.mycel	+	18S		+++
Tricharia ochroleuca		f.mycel	nt	18S		+++
Verjahnia calthae	子嚢菌類	f.mycel	nt	18S		+++
Peziza vesiculosa		f.mycel	nt	18S		+++
Saccharomyces cevisiae	酵母	新鮮	+	18S		+++
藻類						
Gyrodinum aureolum	双鞭毛藻類	新鮮	+++	18S/16S		+++/+++
Hetrocapsa triquetra		新鮮	+++	18S/16S		+++/+++
Scrippsiella trochidea		新鮮	+++	18S/16S		+++/+++
Ceratium stricum		新鮮	+++	18S		+++
Chlorella vulgaris	緑色植物	新鮮	+++	18S		+++
Clamydomonas reinardii		新鮮	+++	18S		+++
Caulacantus ustulata	褐藻類	新鮮	+++	18S		+++
Chrysochromulina polylepis	ハプト藻類 (crysophyceae)	新鮮	+++	18S		+++
植物						
Hordum vulgare (barly)	単子葉類	葉	+++	B15C/trnL		+++/+++
Arabidopsis thaliana	双子葉類	葉	+++	B15C/trnL		+++/+++
脊椎動物						
Perca fluvatilis (perch)	魚類	ep.	+++	D-ループ		+++

[a] A. tumefaciens = Aghrobacterium tumefaciens,

P. hollandica = Prochlorothrix hollandica.

[b] d.fruitb = 乾燥子実体，f.mycelia = 新鮮な菌子体，ep. = ひれ．

[c] 標準的なフェノール／クロロホルム単離に関する大体のＤＮＡ収量

+++: > 80 %, ++: > 10 %, +: > 1 %, nt. = 試験せず

[d] Gen. = ゲノム DNA, Org. = 葉緑体からのオルガネラDNA（藻類および植物）およびミトコンドリア（魚類）

[e] 実施例８に記載したアンプリコン

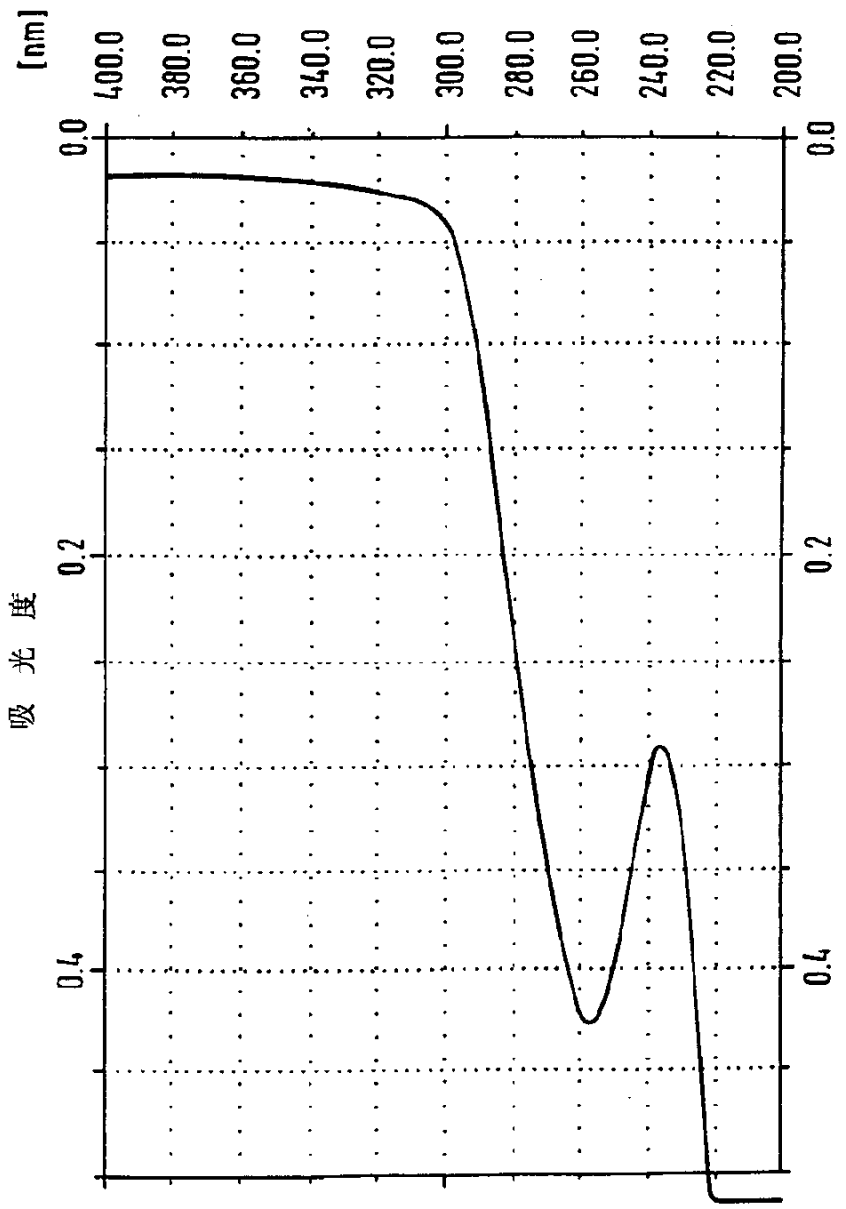
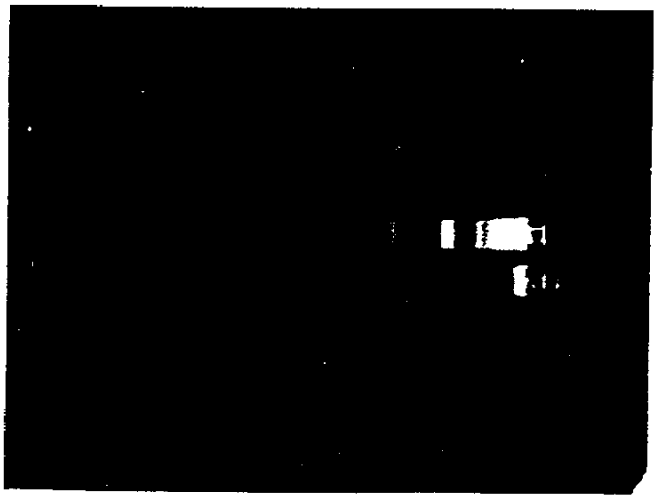
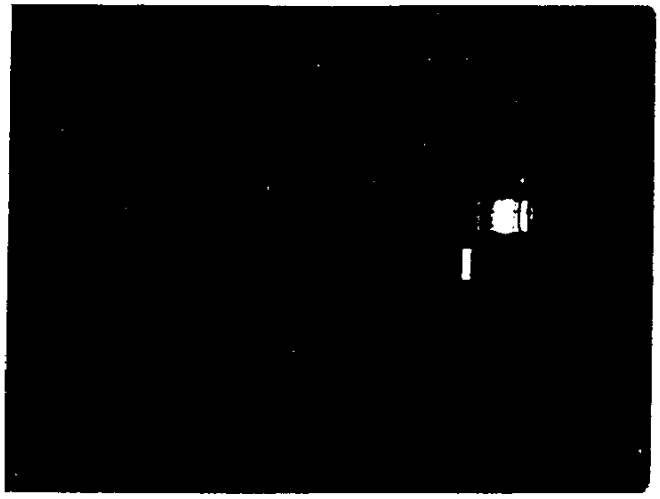

吸光度
[nm]
400.0
380.0
360.0
340.0
320.0
300.0
280.0
260.0
240.0
220.0
200.0
0.0
0.2
0.4
閾値 = 0.100
試料
004
サイクル
15:40
波長
257.6 nm (極大)
236.4 nm (極大)
データ
0.427 ABS
0.292 ABS

FIG. 1



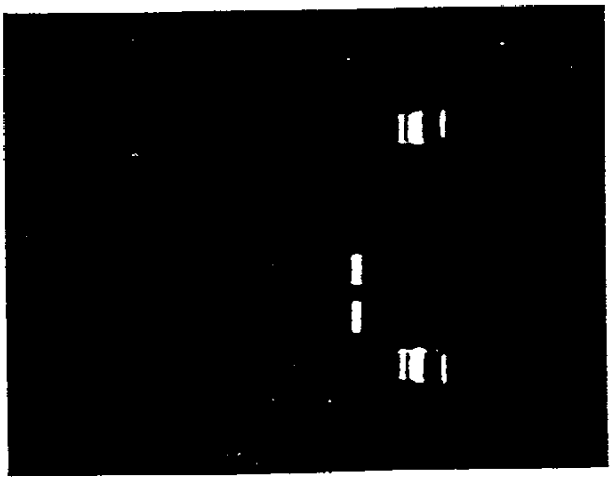
1 2

FIG. 2



1 2 3

FIG. 3



1 2 3 4 5 6

FIG. 4

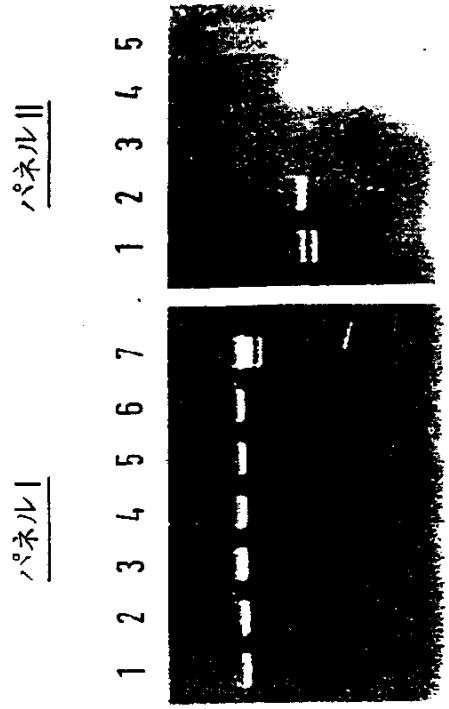


FIG. 5

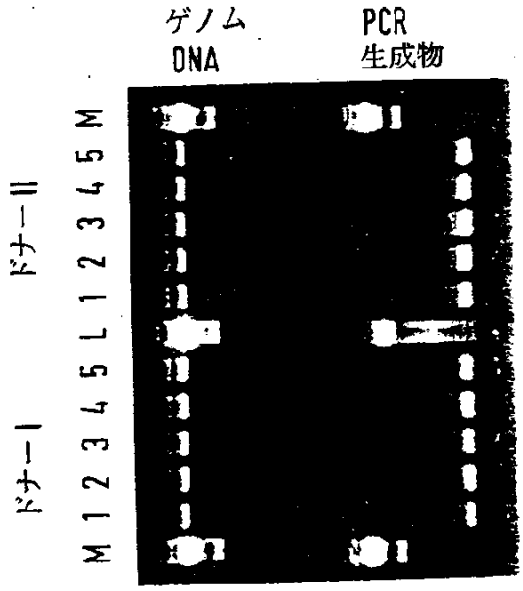

ゲノム
DNA
PCR
生成物
ドナーI
ドナーII
M 1 2 3 4 5 L 1 2 3 4 5 M

FIG. 6

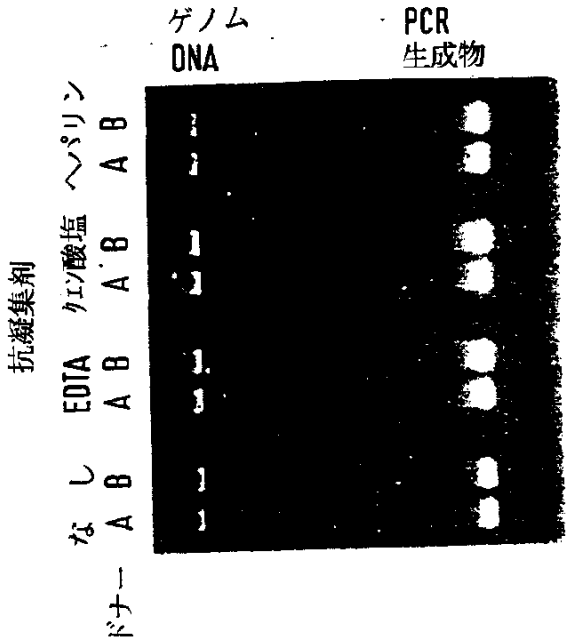

ゲノム
DNA
PCR
生成物
抗凝集剤
なし
EDTA
クエン酸塩
ヘパリン
ドナー
A B A B A'B A B

FIG. 7

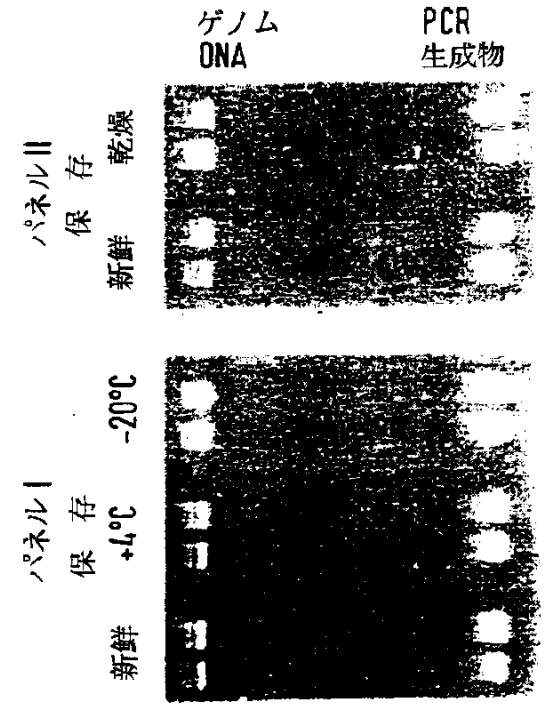

ゲノム
DNA
PCR
生成物
パネルII
保存
新鮮
乾燥
パネルI
保存
+4°C
-20°C
新鮮

FIG. 8

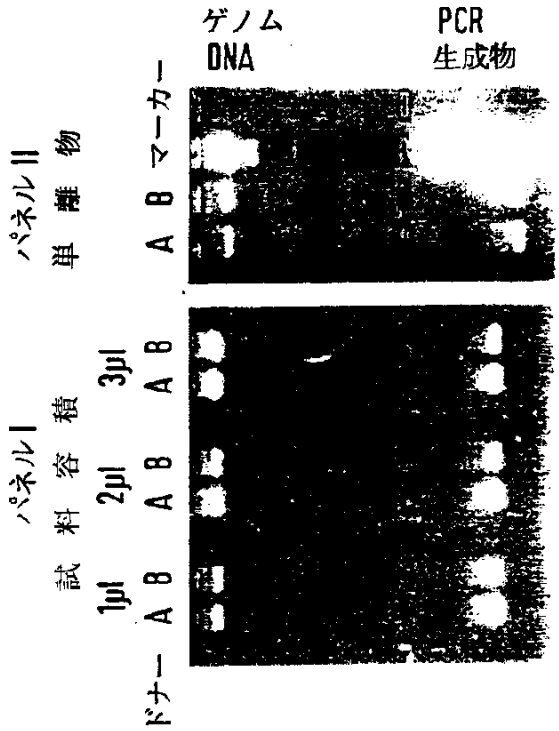

ゲノム
DNA
PCR
生成物
パネルII
単離物
A B マーカー
パネルI
試料容積
1μl 2μl 3μl
A B A B A B
ドナー

FIG. 9

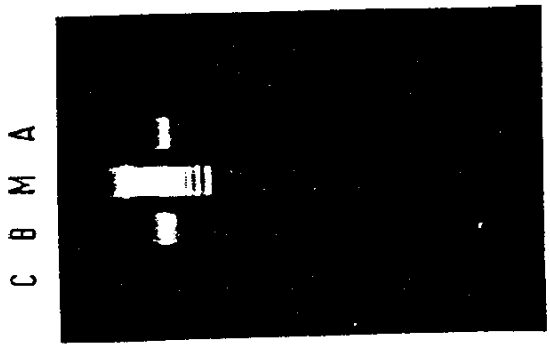


FIG. 10

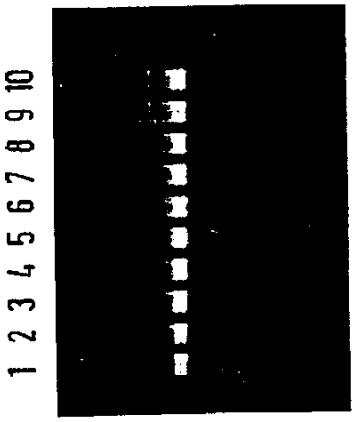


FIG. 11

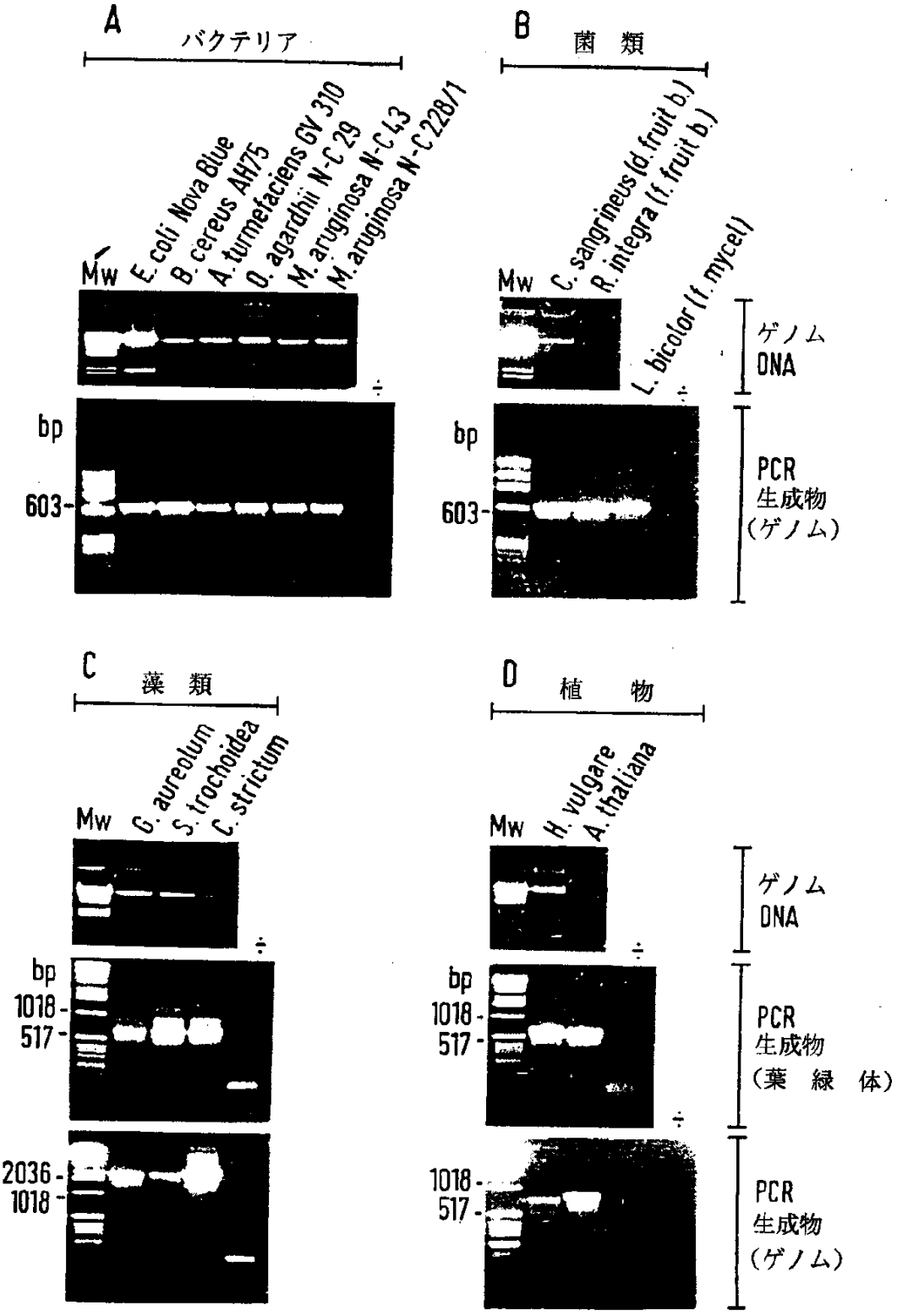

A
バクテリア
Mw
E. coli Nova Blue
B. cereus AH75
A. tumefaciens GV 310
O. agardhii N-C 29
M. aruginosa N-C 43
M. aruginosa N-C 228/1
bp
603
B
菌 類
Mw
C. sangrineus (d. fruit b.)
R. integra (f. fruit b.)
L. bicolor (f. mycel)
bp
603
ゲノム DNA
PCR 生成物 (ゲノム)
C
藻 類
Mw
C. aureolum
S. trochoidea
C. strictum
bp
1018
517
2036
1018
D
植 物
Mw
H. vulgare
A. thaliana
bp
1018
517
1018
517
ゲノム DNA
PCR 生成物 (葉 緑 体)
PCR 生成物 (ゲノム)

FIG. 12

INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
PCT/GB 95/02893

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 25912 (MEDICAL RESEARCH COUNCIL) 23 December 1993 *see the whole patent* ---	1-18
X	JOURNAL OF APPLIED BACTERIOLOGY, vol. 74, 1993, pages 78-85, XP002007385 K. SMALLA ET AL.: "Rapid DNA extraction protocol from soil for polymerase chain reaction mediated amplification" *see the whole article* --- -/--	1-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1996	02. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Marie, A

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INTERNATIONAL SEARCH REPORT

Int. ..onal Application No
PCT/GB 95/02893

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 60, no. 5, 1994, pages 1572-1580, XP002007386 M.I. MORE ET AL.: "Quantitative cell lysis of indogenous microorganisms and rapid extraction of microbial DNA from sediment" *see the whole article* ---	1-18
X	EMBO JOURNAL, vol. 4, no. 4, 1985, pages 913-918, XP002007387 D.A. JACKSON ET AL.: "A general method for preparing chromatin containing intact DNA" *see the whole article* ---	1-18
X	EMBO JOURNAL, vol. 3, no. 8, 1984, pages 1837-1842, XP002007388 P.R. COOK : "A general method for preparing intact nuclear DNA" *see the whole article* ---	1-18
X	EXPERIMENTAL CELL RESEARCH, vol. 190, 1990, pages 294-296, XP002007389 G. KONAT ET AL.: "Rapid isolation of genomin DNA from animal Tissues" *see the whole article* ---	1-18
X	ANALYTICAL BIOCHEMISTRY, vol. 164, 1987, pages 207-213, XP002007390 P.M. GLEE ET AL.: "Methods for DNA extraction from Candida albicans" *see the whole article* -----	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. .onal Application No
PCT/GB 95/02893

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9325912	23-12-93	AU-B-	4343993	04-01-94
		AU-B-	4344093	04-01-94
		WO-A-	9325709	23-12-93

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